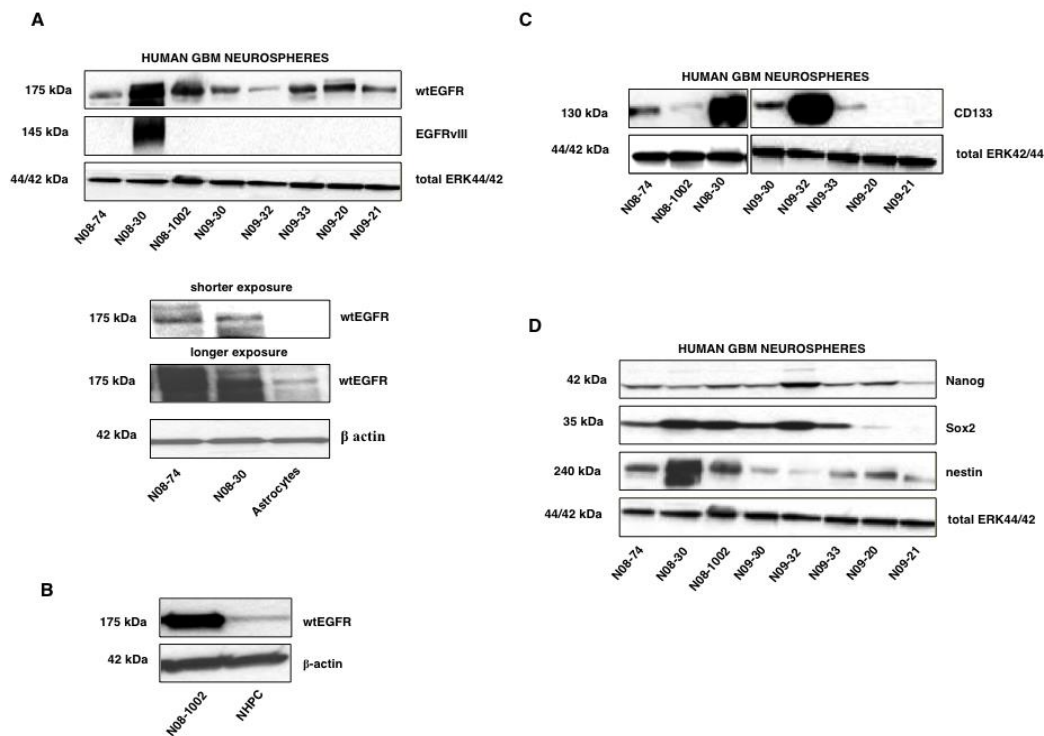
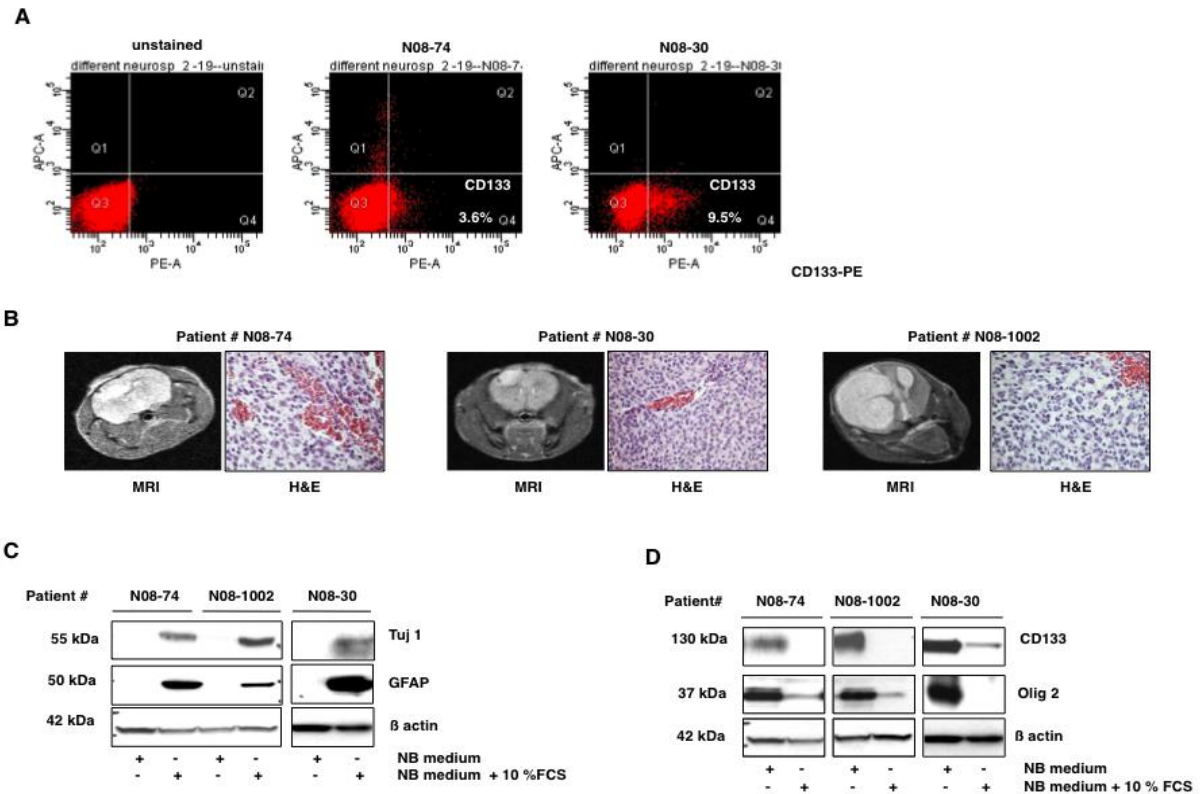


Targeted therapy of glioblastoma stem-like cells and tumor non-stem cells using cetuximab-conjugated iron-oxide nanoparticles

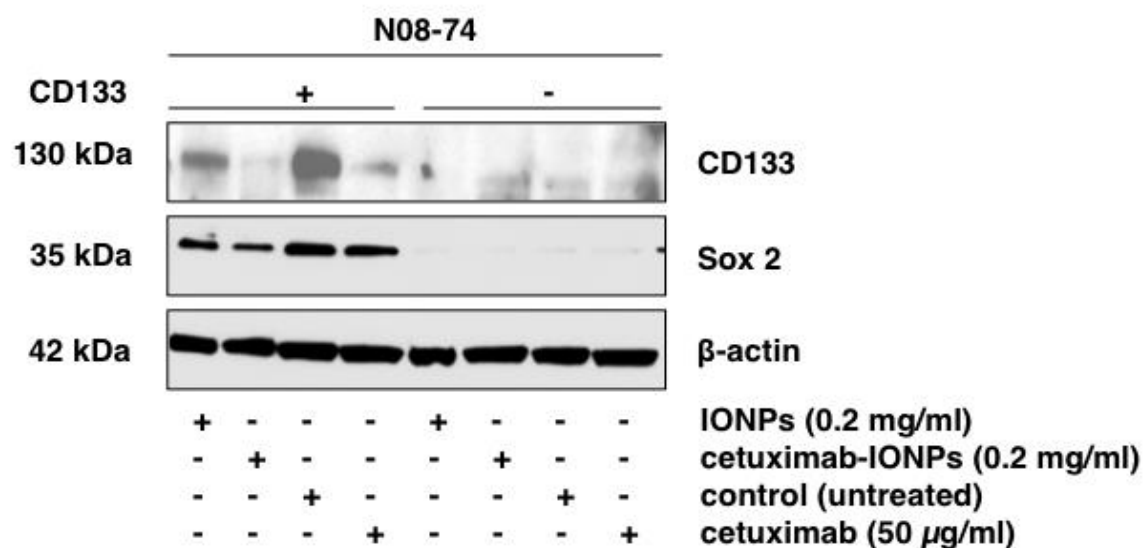
Supplementary Material



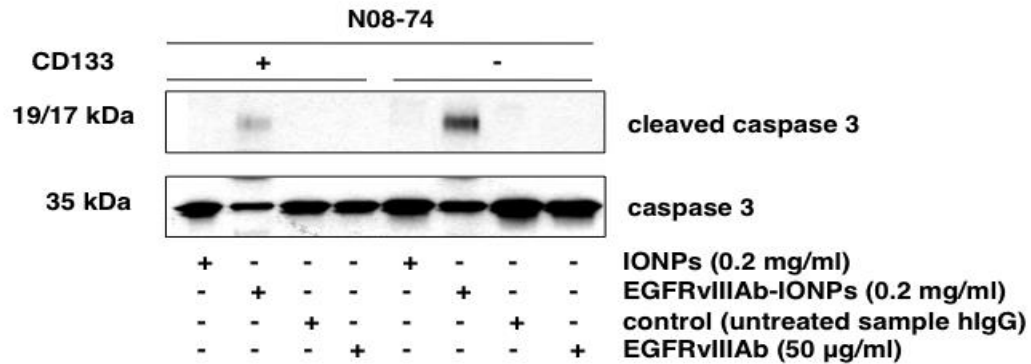
Supplementary Figure 1: Expression of EGFR and molecular profile of GSC-containing human GBM neurospheres. (A) Western blot analysis of EGFR expression in human GBM neurospheres. Only one neurosphere culture (N08-30) is EGFRvIII positive. The lower panel shows that N08-74 and N08-30 neurosphere cultures maintain wtEGFR expression even at late passages (31 and 46, respectively). Normal human astrocytes, used as a control, express low levels of wtEGFR. Short and long exposures are shown. (B) Western blot analysis of EGFR expression in human GBM neurospheres in comparison with neural human progenitor cells (NHPC). Western blot analysis of expression of the stem cell markers CD133 (C), Nanog, Sox2, and Nestin (D). Total ERK was used as internal control. In all experiments, neurospheres were used in early passage.



Supplementary Figure 2: Multilineage differentiation and tumorigenicity of human GBM neurospheres. (A) A representative FACS analysis of human GBM neurospheres N08-74 and N08-30 shows different expression levels of the stem cell marker CD133. (B) MRI and H&E staining of human GBM xenografts generated in athymic nude mice after intracranial implantation of neurospheres N08-74, N08-30, and N08-1002. (C and D) Multilineage differentiation of human GBM neurospheres: Western blotting of neurospheres N08-74, N08-30, and N08-1002, grown in medium with 10% FCS for 11 days, shows multi-lineage differentiation properties as cells became positive for glial (GFAP) and neuronal (Tuj 1) differentiation markers (C) whereas expression of the stem cell markers CD133 and Olig 2 was lost or decreased dramatically (D).

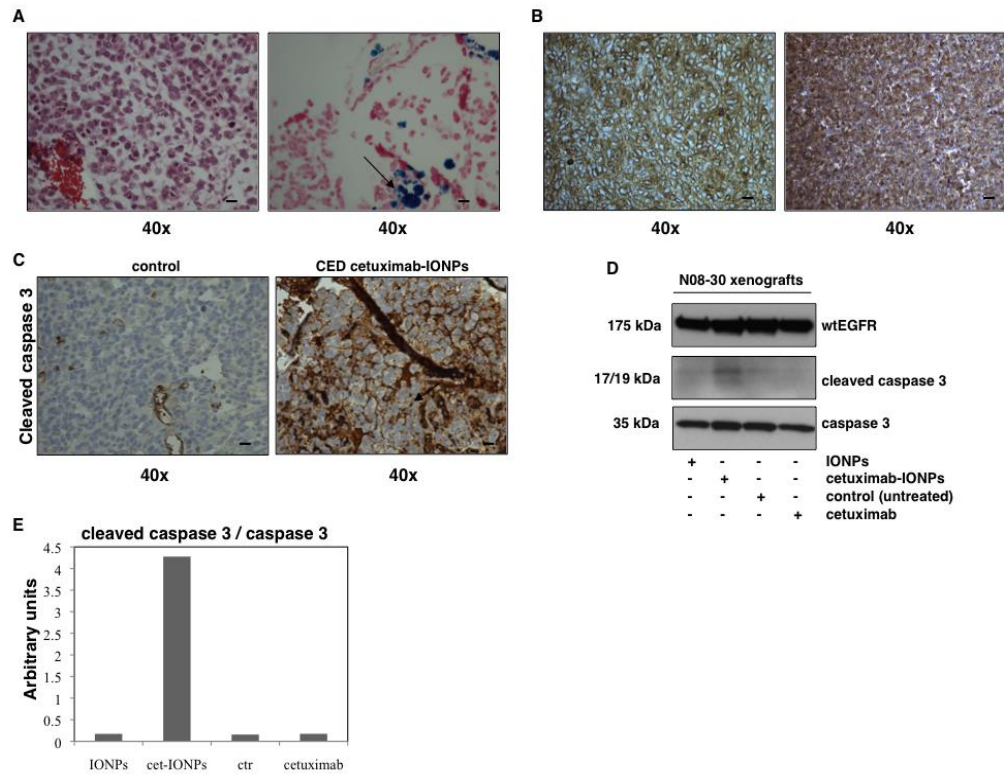


Supplementary Figure 3: Decreased level of stem cell markers in GSCs after treatment with cetuximab-IONPs. GSCs and CD133-negative cells (5×10^5 cells) from neurospheres N08-74 were treated with IONPs (0.2 mg/ml), cetuximab-IONPs (0.2 mg/ml), or cetuximab (50 μg/ml) alone for 3 days and analyzed by Western blotting for expression of stem cells markers CD133 and Sox2. Only cetuximab-IONPs decreased expression of CD133 and Sox2 in GSCs.

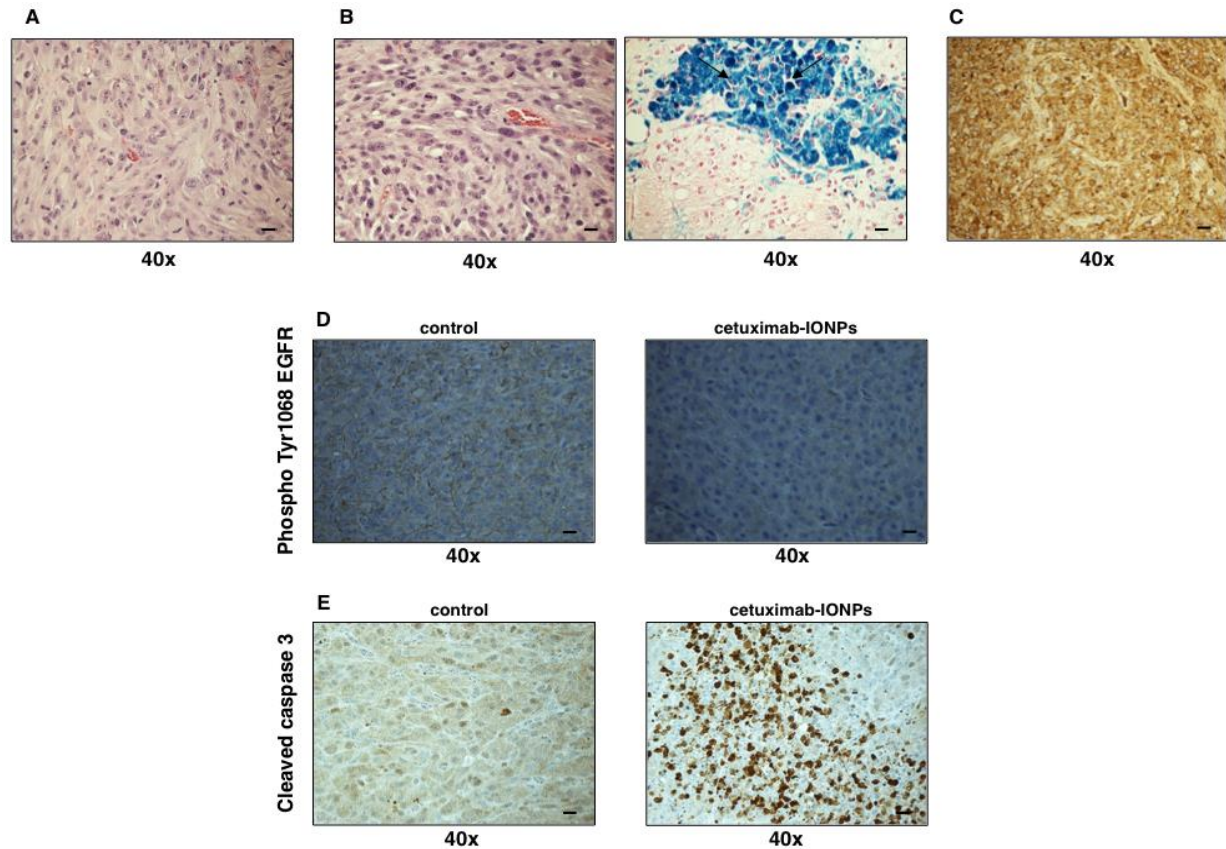


Supplementary Figure 4: Apoptosis in GSCs after treatment with EGFRvIIIAb-IONPs.

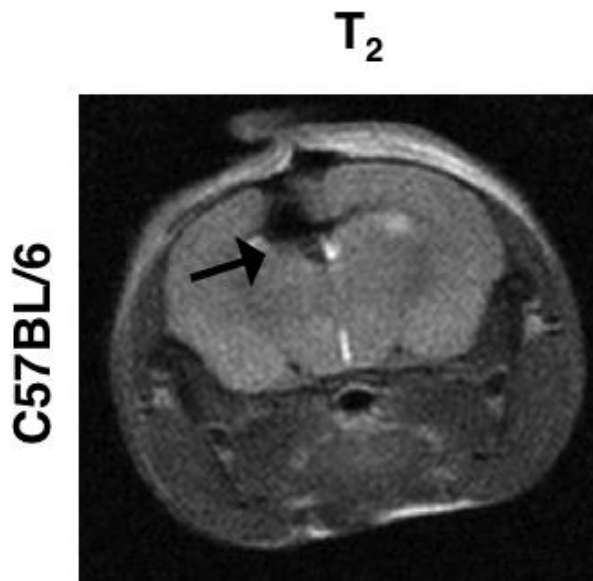
GSCs and CD133-negative cells (5×10^5 cells) from neurospheres N08-74 were treated with IONPs (0.2 mg/ml), EGFRvIIIAb-IONPs (0.2 mg/ml), or EGFRvIIIAb (50 µg/ml) alone for 3 hs and analyzed by Western blotting for expression of cleaved caspase 3 and caspase 3. Only EGFRvIIIAb-IONPs activated caspase 3 cleavage in both GSCs and GBM CD133-negative cells.



Supplementary Figure 5: Analysis of a representative human GBM xenograft derived from GSC-containing N08-30 neurospheres in the brain of athymic nude mice after CED treatment with cetuximab-IONPs. (A, left) H&E staining confirms the human GBM xenograft (magnification 40x). **(A, right)** Prussian blue staining following CED of cetuximab-IONPs confirms intra-tumoral presence of cetuximab-IONPs (black arrow, magnification 40x). Immunostaining of a human GBM xenograft in an athymic nude mouse for wtEGFR **(B, left)**, EGFRvIII **(B, right)**, and cleaved caspase 3 (control mouse and mouse after CED treatment with the cetuximab-IONPs) **(C)**, (magnification 40x). **(D)** Lysates of xenografts from N08-30 human GBM neurospheres were analyzed by Western blotting for cleaved caspase 3, caspase 3, and wtEGFR expression. Only cetuximab-IONPs induce cleavage of caspase 3. **(E)** Densitometric analysis confirms a significantly higher ratio of cleaved caspase 3/full length caspase 3 in the cetuximab-IONP treated GBM xenografts ($P<0.005$).



Supplementary Figure 6: Analysis of a representative human GBM xenograft derived from U87MGwtEGFR cells in the brain of athymic nude mice after CED treatment with cetuximab-IONPs. (A) H&E staining confirms the human GBM xenograft 5 days after injection (magnification 40x). (B, left) H&E and (B, right) Prussian blue staining after CED of cetuximab-IONPs confirms intra-tumoral presence of the cetuximab-IONPs (black arrow, magnification 40x). Immunostaining for wtEGFR (C), phospho-EGFR Y1068 (D), and cleaved caspase 3 (E) of intracranial human U87MGwtEGFR GBM xenografts in control and cetuximab-IONP CED-treated athymic nude mouse (magnification 40x). Less phosphorylation and increased levels of cleaved caspase 3 were found in cetuximab-IONP treated xenografts.



Supplementary Figure 7: Photomicrograph of an immunocompetent mouse brain after CED of cetuximab-IONPs. T_2 weighted MRI after CED of cetuximab-IONPs, day 1 (cetuximab-IONPs, black arrow) shows no hemorrhage in the brain.